Laboratory Studies on The Efficacy of *Annona Muricata* Seed Crude Extract in Protecting Stored Rice Grain Against *Sitophilus Zeamais* (Coleoptera: Curculionidae)

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ABSTRACT

The study was conducted to determine the efficacy of *Annona muricata* seed crude extract against *Sitophilus zeamais* in protecting stored rice grain. The seed crude extract were admixed with the rice grain at 0 (control), 0.025, 0.05, 0.1, 0.2, 0.4% (v/w). The result showed all concentrations tested caused significance effects on adult mortality, number of progeny and percent weight loss. The 0.4% of *A. muricata* seed crude extract concentration caused the highest mortality (100%), no progeny produced, no weight loss and caused the fastest mortality (about 100% at 3 days after treatment). The rice grain treated with 0.2% concentration caused 86% adult mortality, 32.4 of progeny produced and 3.28% of weight loss. The moderate effect was shown at 0.1% concentration (67% of adult mortality, 68 of progeny produced and 4.25% weight loss). The lowest effects of crude extract tested was shown at 0.05 and 0.025% concentration of seed crude extract with about 34% adult mortality, 254.6 and 286 progeny produced, 8.97 and 11.24% of weight loss. The crude extract of *A. muricata* at 0.4% concentration provided good protection for rice grain agains *S. zeamais*.

Key words: Efficacy, *Annona muricata*, seed crude extract, *Sitophilus zeamais*, rice grain

Introduction

In Indonesia, rice grains are continuously stored in huge quantity for food security. It is infested by many insect pests, especially *S. zeamais*. The rice weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) is one of the major primary pests of grain. (Sidik and Pranata, 1988). For controlling of insect pest in rice storage, the application insecticide seemed to be necessary. The insecticides that commonly used in storage are fumigants and residual grain protectants. However, over relying on pesticides may result in insecticide resistance development, contamination of toxic residues in stored product grain, hazards when handling the toxic compounds as well as polluting the environment (Yusof and Ho, 1992; Golob et al., 1999). There is a need to find the alternative control method such as using botanical insecticides to substitute the synthetic chemicals. Insecticidal property Annonaceae seed extract has been reported by some searcher. It could affected leafhopper *Nilaparvata lugens* and *Spodoptera litura* (Mariapan and Saxena ,1984; Leatemia and Isman, 2004). This study evaluated the efficacy of *A. muricata* seed crude extract against *S. zeamais* on rice grain. *Azadirachta indica* (neem) seed crude extract also used to compare the efficacy because neem is the most popular and commercially produced pesticide (Novizan, 2002).

Material and Methods

The initial population of *S. zeamais* was obtained from rice grain in the open market. The insect culture was maintained on rice grain as growth medium. The rice grain and all apparatus or equipments were sterilized by heating at 60^{o}C for 1 h to protect stock culture from its natural enemies (insect, mites and pathogen).The *S. zeamais* was

cultured for 3th generations before used in the experiment to avoid external factors and to acclimatize the stock with the laboratory condition. Fifty of 50 adults were fed on 150 g rice grain filled up in a transparent plastic cup (7 cm high, 9.5 top and 8.5 cm bottom diameter, respectively) for 1 week. They were then removed and the rice grains were incubated at 28 ± 2ºC and 75 ± 10% R.H. until adult emergence (ca. 4 weeks).

The *A. muricata* and *A. indica* seeds were collected from around Medan City, Indonesia in February 2015. The seeds collected from mature fruit were hulled to get the kernel, and ground with electric grinder to obtain the powder for extraction process using soxhlet extractor. The 50 g of powdered materials was placed in a paper timble. Then 200 ml acetone was poured in to the receiving flask. The process extraction took about 8 h. Crude extract obtained after complete removal of the solvents with vacuum evaporation at temperature <40ºC.

The application of crude extract on rice grain was done at 0.025, 0.05, 0.1, 0.2 and 0.4% (v/w) concentrations. Each concentration was diluted in 2 ml acetone and poured into the 100 ml of flask containing 100 g rice grain. The flask was shaken manually and the coated rice grains were then taken out and air-dried for 30 min. A total of 20 g rice grains were put into each plastic cup. Rice grains treated with only acetone were used as Control. Twenty 5-7 days old adults were released in each cup and covered with a piece of muslin cloth held by rubber band to prevent adults from escaping. Each treatment was replicated five times. Mortality was recorded everyday for three weeks after treatment (Sighamony et al., 1985).

The experiments were conducted at 28 ± 2ºC and 75 ± 10% R.H. arranged following CRD with 5 replications. Data (mortality, progeny, and weight loss) analyzed one-way ANOVA. Data of *S. zeamais* mortality were transformed using arcsin $\sqrt{x}$, number progeny production was normalized using log 10 *x*+1, while $\sqrt{x+0.5}$ for weight loss (Gomez & Gomez, 1984). Data were subjected to analysis of variance and where significant differences existed, treatments means were compared at 0.05 significant level using DMRT Test.

Result and Discussion

There was a significant effect between *A. muricata* seed crude extract tested on the percent *S. zeamais* mortality, progeny produced and weight loss. The effect of different *A. muricata* crude extract concentration on percentage of *S. zeamais* mortality, progeny produced and weight loss as presented in Table 1.

Table 1. Effect of *A. muricata* seed crude extract on adult mortality, number of progeny production and weight loss

Concentration (%)	Mortality (%)	Progeny produced	Weight loss %
0	10 e	316,2 a	10,43 ab
Am 0.025	34 d	286 ab	11,24 a
Am 0.05	34 d	254,6 abc	8,97 abc
Am 0.1	67 c	68 e	4,25 e
Am 0.2	86 b	32,4 f	3,28 ef
Am 0.4	100 a	0 g	1,31 g
Ai 0.4	35 d	165 abcd	8,82 abcd

Means in a column followed by different letters are significantly different at P=0.05 by DMRT Test. Am = *Annona muricata.* Ai = *Azadirachta indica*

The result showed that *A. muricata* seed crude extract caused significance effect on adult mortality, number of progeny produced and weight loss. The 0.4% of *A. muricata* seed crude extract concentration caused the highest mortality (100%), no progeny produced, no weight loss. Whilst, similar concentration with *A. indica* caused significance difference (35% of mortality, 165 of progeny produced and 8.82 of weight loss). The toxicity of *A. muricata* seed crude extract against *S. zeamais* due to the presence of acetogenin in the seed. Santos and Sant'Ana (2001) and Isman (2006) reported that the species such as *A. muricata* had the Annonaceous acetogenins, a class of natural compound with a wide range of biological activities. There was progeny production and weight loss when rice grain treated at 0.2% concentration of *A. muricata* seed crude extract. At this concentration, the mortality 80% occurred after about 2 weeks after application (Figure 1), hence, the life weevil still attack the rice, copulated and produced the progeny and weight loss. The other concentrations showed less effect, accordingly with the less of concentration applied.

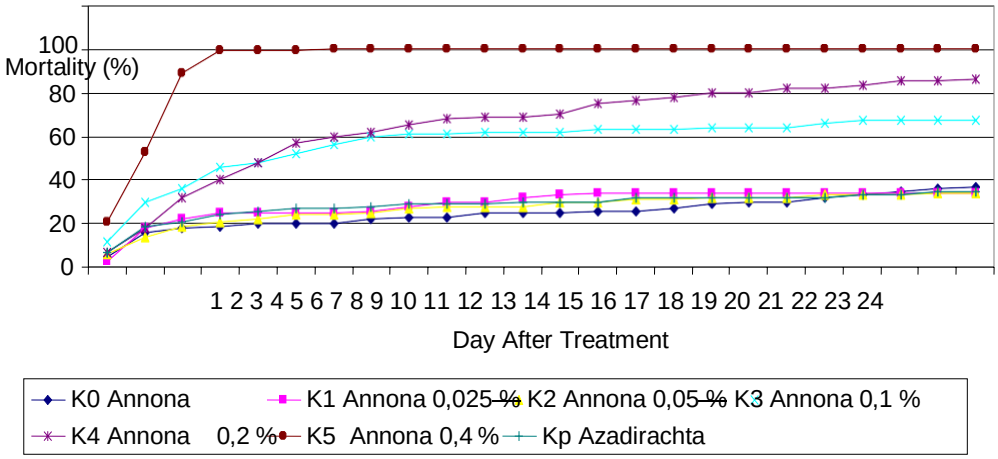

Figure 1. Cumulative percent mortality within 24 days of *S. zeamais* adult fed on the rice grain treated with *A. muricata* seed crude extract

Although the effectiveness of neem tree has been reported by many authors (Pandey et al., 1986; Makanjuola, 1989), in the present study, the crude seed extract of *A. indica* caused significantly lower mortality on *S. zeamais* than crude seed extract of *A. muricata*. The different may be due to the maturity of seeds.

Conclusion

Crude extracts of *A. muricata* seed seem to be potential to be used as botanical insecticides and further study needed to conducted against *S. zeamais*.

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